

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 1 595 536 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:

16.11.2005 Bulletin 2005/46

(51) Int Cl.7: **A61K 31/122**, A61P 31/00,

A61P 7/00, C07C 50/32

(21) Application number: **03815939.8**

(86) International application number:

PCT/CN2003/000138

(22) Date of filing: **21.02.2003**

(87) International publication number:

WO 2004/073699 (02.09.2004 Gazette 2004/36)

(84) Designated Contracting States:

**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IT LI LU MC NL PT SE SI SK TR**

Designated Extension States:

AL LT LV MK RO

(72) Inventor: **WANG, Feixin**

100012 Beijing (CN)

(71) Applicant: **Beijing JBC Chinese Traditional**

Medicine Science

and Technology Develop Co. Ltd.

Beijing 100012 (CN)

(74) Representative:

Hoarton, Lloyd Douglas Charles et al

Forrester & Boehmert,

Pettenkoferstrasse 20-22

80336 München (DE)

(54) **PHARMACEUTICALS COMPRISING SHIKONINS AS ACTIVE CONSTITUENT**

(57) The object of the invention is to provide medicaments containing Shikonin(include shikonin and alkannin) compounds and salts thereof, which are used for treatment or prevention of microorganism infection

in human body, inflammation, malignant tumor, hemorrhage, hematopathy, and autoimmune disease.

EP 1 595 536 A1

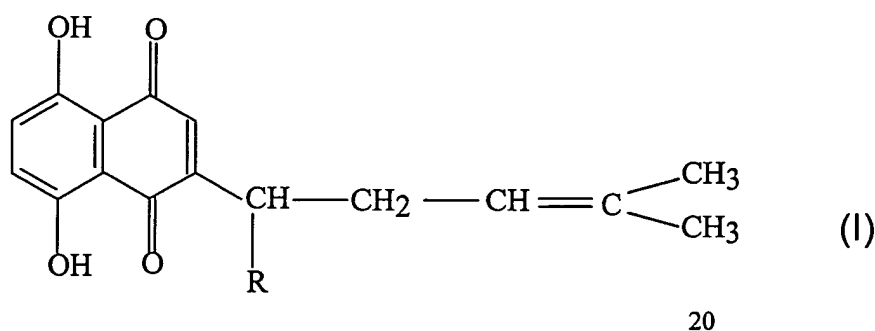
Description

Technical Field

[0001] The present invention is involved in a medicament containing Shikonin compounds or its salt, specially the medicament containing Shikonin compounds or its salt as an active component designing to prevent and treat microorganism infection in human body, inflammation, tumour, hemorrhage, hematopathy, and autoimmune disease.

Technical background

[0002] Shikonin compounds are an opened compound reported in literatures (Lin Zhibin, et al, P101-105, Issue 2, Volume 12, 1980, JOURNAL OF BEIJING MEDICAL UNIVERSITY), therein, the Shikonin compounds have the following general formula structure:

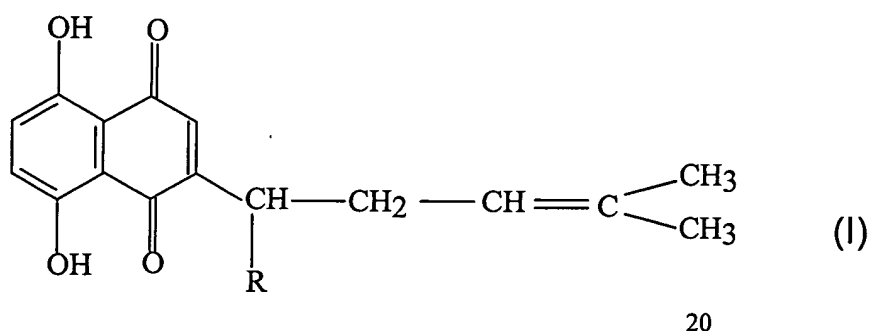


[0003] Shikonin compounds are insoluble in water but freely soluble in oil, alcohol or ethers, abstracted from Boraginaceae plants: *Lithospermum erythrorhizo* Sieb. et Zucc.; *Arnebia euchroma* (Royle) Johnston. It's known that the Zicao mixture extract has some functions such as anti-inflammation, but it's just mixed in the form of mixture extract when medicament delivery. While it has not been reported yet that the Zicao quinone compound extracted from plant Shikonin and artificial or biosynthetic Shikonin quinone compounds are designed to manufacture medicaments in single compound or combination of several compounds, particularly for prevention and treatment of microorganism infection in human body, inflammation, tumour, hemorrhage, hematopathy and autoimmune disease.

Content of invention

[0004] Therefore, the present invention is designed to provide a single compound or several compounds separated from Zicao extract to manufacture a medicament for prevention and treatment of microorganism infection in human body, inflammation, tumor, hemorrhage, hematopathy and autoimmune disease.

[0005] The present invention provides a medicament to prevent and treat microorganism infection in human body, inflammation, tumour, hemorrhage, hematopathy, and autoimmune, which comprises one to five Shikonin compounds or its salt represented in the following Formula (1)



wherein, R is a group selected from a group composed of H(deoxyshikonin), OH(Shikonin), $(\text{CH}_3)_2\text{C}=\text{CHC}(\text{O})\text{O}-$ (β , β -dimethylacry), $\text{CH}_3\text{C}(\text{O})\text{O}-$ (Acetylshikonin), $(\text{CH}_3)_2\text{C}=\text{C}(\text{CH}_3)\text{CH}_2\text{C}(\text{O})\text{O}-$ (teracrylshikonin), $(\text{CH}_3)_2\text{COHCH}_2\text{C}(\text{O})-$ (β -hydroxyisovalerylshikonin), $(\text{CH}_3)_2\text{C}[\text{OC}(\text{O})\text{CH}_3]\text{CH}_2\text{C}(\text{O})\text{O}-$ (β -acetoxyisovalerylshikonin); and preferably, said medicament contains 1 to 3 compounds selected from Shikonin, β , β -dimethylacrylshikonin and Acetylshikonin; more preferably, said medicament contains β , β -dimethylacrylshikonin and/or Acetylshikonin; the most preferably, said medicament contains β , β -dimethylacrylshikonin. The salts of Shikonin compounds in this invention include the salts of alkali metals, alkaline earth metal and ammonium, etc thereof.

[0006] The medicament according to this invention contains one or more compound(s) of the Shikonin compounds as raw medicaments, of which the purity of single compound is 80% or more, and the preferable purity is 90% or more. When the medicine contains a combination of several compounds, the effective components thereof is 70% or more.

[0007] If necessary, the invented medicament can further contain other active components. There is no extraordinary restriction to the other active components, which the technologist can select properly in accordance to the existing technology.

[0008] The content scope of Shikonin compounds in the invented medicament ranges from 0.0001% to 75% (weight percent), which can be selected properly according to different preparation as well as symptoms of disease. When being used in human body, the daily consumption of the mentioned Shikonin compounds can be controlled between 10i g-20g, the preferable one is 100i g-10g, and the more preferable one is 1 mg-8g, and the best one is 5g, which can be selected properly in accordance to the different status such as age, weight and state of illness for different sufferers. It can be used for a single time or several times. The invented medicament can be delivered in oral administration, external application, injection, inhalation or skin penetration.

[0009] The Shikonin compounds in the invention can be used for prevention and treatment of microorganism infection including pathogenic Gram-positive micrococcus, such as staphylococcus, streptococcus pneumonia, staphylococcus epidermidis and enterococcus; pathogenic Gram-negative micrococcus such as Klebsiella pneumonia ozaenae, Serratia marcescens, Stenotrophomonas maltophilia; anaerobic or little aerobic pathogen such as Helicobacter pylori; Eumycetes such as deep and superficial eumycetes; Leuconostoc spp, aspergillus fumigatus, cryptococcus, dermatophyte, krusei leuconostoc spp, Cercospora punicae, etc; and all kinds of mycoplasma infection particularly the mycoplasma infection of the respiratory system; virus such as hepatitis B virus, cold virus, herpes virus and HIV virus, etc.

[0010] The Shikonin compounds can be used for prevention and treatment of inflammation of human body, including phlebitis, vascular purpura, colpitis and edema, etc.

[0011] It also can be used for prevention and treatment of hemorrhage and hematopathy in human body, for instance, burning, scalding, all kinds of dermatitis, septicemia hemophilia, primary thrombocythemia, leukaemia, etc.

[0012] It also can be used for prevention and treatment of tumour especially malignant tumor, for instance, ascitic type tumour: liver cancer, L1210; solid tumour: W256, S180, gastric cancer 823, squamous cell carcinoma 109, Lewis lung cancer, etc.

[0013] The medicament containing Shikonin compounds in the present invention can be used for prevention and treatment of autoimmune disease of human body, i.e. promoting human body's functions of nonspecific immunity and idiosyncratic cell-mediated immunity through improving the function of immune response of T lymphocytes.

[0014] Therefore, the medicament according to the present invention are available for respiratory system, digestive system, urinary system, reproductive system, blood system, circulating system and skin or mucous membrane in human body.

mode of carrying out the invention

[0015] The following text gives detailed description on the manufacture of pharmaceutical preparation containing Shikonin compounds and pharmacodynamics experiments of the present invention, but the protection scope of the present invention is not limited to this.

Preparation example 1

[0016] Shatter 2 kg Arnebia euchroma(Royle)Johnst. components, make abstraction with petroleum ether till residue of Arnebia euchroma(Royle)Johnst is colorless, recover the solvent and get 80 g dark red paste. Separate the paste through silica gel H-column liquid chromatography and carry out gradient elution with 1%-20% ethyl acetate-petroleum ether, and then get 7 monomers of Shikonin compounds stated above, i.e. 2.944 g deoxyshikonin(yield is 3.68£¥), 0.712 g Shikonin (yield is 0.89£¥), 29.024 g β , β -dimethylacrylshikonin (yield is 36.28£¥), 13.27 g Acetylshikonin(yield is 16.59£¥), 6.032 g teracrylshikonin (yield is 7.54£¥), 0.776 g β -hydroxyisovalerylshikonin(yield is 0.97£¥), 0.792 g β -acetoxyisovalerylshikonin(yield is 0.99£¥). By high-pressure liquid chromatography, all purity is over 90%.

Preparation example 2

[0017] Shatter 2 kg *Arnebia euchroma*(Royle)Johnst. components, go through 20-40 meshes and get 70 g red ointment by CO₂-supercritical extraction. Separate the cream by high-pressure liquid preparative chromatography (Germany Knauer K1001 type) with preparative column: silica gel H 10i m 50X300mm and carry out gradient elution with 1%-20% ethyl acetate-petroleum ether, and then get 7 red monomers of Shikonin compounds stated above, i.e. 3.486 g deoxyshikonin(yield is 4.98£¥), 0.707 g Shikonin (yield is 1.01£¥), 30.877 g β , β -dimethylacrylshikonin (yield is 44.11£¥), 15.869 g Acetylshikonin (yield is 22.67£¥), 6.034 g teracrylshikonin (yield is 8.62£¥), 0.91 g β -hydroxyisovalerylshikonin (yield is 1.30£¥) and 0.77 g β -acetoxisovalerylshikonin(yield is 1.10£¥). By high-pressure liquid chromatography, all purity is over 90%.

Example 1

[0018] Manufacture troche with single or several combination of the above 7 compounds according to the way widely known by technical personnel of the field, of which the troche with 10% -70% Shikonin compounds can be made according to actual demand.

[0019] Take 100 g β , β -dimethylacrylshikonin got in Manufacture example 1 or Manufacture example 2, 100 g nucleated fiber, 30 g magnesium stearate, and 4 g hydroxypropyl methyl cellulose under the aseptic operation conditions. 0.5 g tablet can be made according to widely known troche made technology and equipment.

Example 2

[0020] Manufacture 0.5 g tablet with 100 g combination of Shikonin compounds got in Manufacture example 1 or Manufacture example 2 (combination proportion of Shikonin, β , β -dimethylacrylshikonin and Acetylshikonin is 1:1:2) and the left in the same way as Implementation example 1.

Example 3

[0021] Manufacture the ointment of the above 7 Shikonin compounds according to the way widely known by technical personnel of the field, of which the ointment with 0.0001%-10% Shikonin compounds can be made according to actual demand. Under the aseptic operation conditions, take 0.5 g Shikonin compounds got in Manufacture example 1 or Manufacture example 2 (combination proportion of deoxyshikonin, Shikonin, β , β -dimethylacrylshikonin Acetylshikonin and β -hydroxyisovalerylshikoninis 0.7:1:1:2:0.5), 80 g vaseline, 10 g liquid paraffin and 10 g anhydrous lanolin and equably triturate them into products in separate bags for external use. This ointment also can be made into patch for skin penetration in a way widely known by technical personnel of the field.

Example 4

[0022] Manufacture the injection of the above 7 Shikonin compounds according to the way widely known by technical personnel of the field. Under the aseptic operation conditions, take 0.5 g β , β -dimethylacrylshikonin got in Manufacture example 1 or Manufacture example 2, 400 ml propylene glycol, 100 ml ethanol, 20 ml tween-80 and 15 ml benzyl alcohol, make them fully dissolved and add water up to 1,000 ml. After mixing well, bottle them to be injection product. The following description is on test result of effect of the medicament containing Shikonin compounds.

(1) Dispensation of the drug

[0023] Respectively take 5.0 mg Shikonin, β , β -dimethylacrylshikonin, Acetylshikonin got in Manufacture example 1 or Manufacture example 2. Make the medicament dissolved in 1 ml DMSO. After diluting by 50 times with RPMI-1640 culture medium, separately pack them and further dilute into the following concentration: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 (i g/ml).

(2) Sensitivity test of the drug

[0024] Separately pack the medicament at above concentration into the orifice plate and vaccinate with all bacterial strains at a density of 10³-10⁶.

[0025] The test result indicates that Shikonin, β , β -dimethylacrylshikonin and Acetylshikonin have high sensitivity to Gram-positive staphylococcus aureus and the MIC is 0.391-12.5i g/ml; for Gram-negative pathogen, the MIC of pneumobacillus is 0.391-6.25i g/ml and that of some bacterial strains is 12.5-50i g/ml; most isolates of bacillus pro-

digiosus and most bacterial strains of *Stenotrophomonas bacilli* have a MIC of 0.391 - 3.1251 g/ml. Therein, they are especially effective to *Stenotrophomonas bacilli* and the MIC is 0.391-0.781 g/ml while that to berberine is 8-32 g/ml, i.e. it is obviously better than berberine. For bacteroid, especially *Bacteroides fragilis*, the MIC is 0.391-6.25 g/ml; they are highly sensitive to *Helicobacter pylori* and the MIC is 0.391 - 0.781 g/ml.

[0026] Additionally, the result of α , β -dimethylacrylshikonin invitro antifungal test indicates that the MIC for *Candida* and *Cryptococcus* is 2.08-33.3 g/ml and MIC₉₀ is 33.3 g/ml; for fluconazole the MIC is 0.125-64 g/ml and MIC₉₀ is 69 g/ml; to dermatophyte the MIC is 4.16-8.32 g/ml with MIC₉₀ of 4.16 g/ml while the MIC of fluconazole to most bacterial strains of dermatophyte is 32-64 g/ml with MIC₉₀ of 64 g/ml. There are obvious differences in both of them. Furthermore, α , β -dimethylacrylshikonin has good inhibitory effect to *C. krusei* that resists fluconazole and the MIC is 8.32-16.6 g/ml, and for *Pseudallescheria boydii* that is insensitive to most antifungal medicaments like fluconazole, the MIC is 4.16-8.32 g/ml. Besides, the MIC of Acetylshikonin against *Cryptococcus neoformans* is 3.90625 g/ml, against red trichophyton is 0.90625- 62.5 g/ml; the MIC of α , β -dimethylacrylshikonin against *Aspergillus fumigatus*, *Cryptococcus* and red trichophyton is 3.0625- 250 g/ml. Therefore, Shikonin compounds are broadspectrum and effective antifungal drug.

[0027] In addition, Shikonin compounds of the invention have a MIC of over 200 g/ml on the microbes like *Lactobacilli* and *Bifidobacterium* beneficial for human body. Therefore, the above data indicates that medicaments with Shikonin compounds in the invention are sensitive to pathogenic microorganism but insensitive to microbes beneficial for human body.

[0028] From the comparative experiment between the mixed extraction from Zicao and 1-3 kinds of Shikonin compounds, it is observed that the medicaments containing Shikonin compounds are obviously better than mixed extraction from Zicao; the results of MIC (g/ml) are shown in Table 1.

Table 1

| Name of bacterial strain | A | B | C |
|---|------|--------|--------|
| <i>Staphylococcus epidermidis</i> | 12.5 | 0.391 | 0.7812 |
| <i>Serratia marcescens</i> | 25 | 0.781 | 3.125 |
| Bacteroid | >200 | 0.391 | 0.391 |
| <i>Candida albicans</i> | 500 | 3.9062 | 250 |
| Note: A is mixed extraction from Zicao B is α , β -dimethylacrylshikonin C is mixture of Shikonin compounds (the mixture ratio of Shikonin, α , β -dimethylacrylshikonin and Acetylshikonin is 1:1:2) | | | |

[0029] The experiment of Shikonin, α , β -dimethylacrylshikonin and Acetylshikonin's bacteriostatic effect on *Mycoplasma pneumoniae* shows that, their MIC for *Mycoplasma pneumoniae* are respectively 3.751 g/ml, 2 g/ml and 7.819 g/ml, equivalent to the inhibitory effect of 0.1925 g/ml erythrocine.

[0030] The following table shows the test results for using Shikonin compound ointment made in Implementation Example 3 as external remedy for treating some disease.

Table 2

| Cases | Number of subjects | Effective percentage | Cured percentage | Days of treatment | Medicament Delivery route | Note |
|-----------------|--------------------|----------------------|------------------|-------------------|----------------------------------|---|
| Burn & scalding | 300 | 100% | 100% | 6-20 | Direct delivery at affected part | 92 people scalded, 186 second degree superficial burns, 114 deep second degree and third degree burns |

EP 1 595 536 A1

Table 2 (continued)

| Cases | Number of subjects | Effective percentage | Cured percentage | Days of treatment | Medicament Delivery route | Note |
|----------------------------|--------------------|----------------------|------------------|-------------------|----------------------------------|---|
| Hemorrhoids | 117 | 100% | 97.4% | 15 | Direct delivery at affected part | Recurrence in three cases after half a year |
| Herpes zoster | 98 | 100% | 100% | 3-7 | Direct delivery at affected part | Polyinosinic-polytidylin acid is used in 12 cases |
| Cervical erosion | 80 | 100% | 100% | 10-20 | Vagina delivery | |
| Children's nosebleed | 257 | 99.6% | 72.8% | 15 | Nasal cavity delivery | |
| Verruca plana | 100 | 96% | 81% | 10-30 | Direct delivery at affected part | |
| Chronic prostatitis | 40 | 82.5% | 57.5% | 10-20 | Anus delivery | |
| Acne | 50 | 92% | 60% | 15 | Direct delivery at affected part | |
| Bedsore | 30 | 100% | 100% | 7-21 | Direct delivery at affected part | |
| Eczema rhagadiforme | 98 | 94.9% | 66.3% | 10-30 | Direct delivery at affected part | |
| Verruca acuminata | 55 | 100% | 100% | 5-35 | Direct delivery at affected part | |
| Infantal diaper dermatitis | 208 | 100% | 100% | 2-6 | Direct delivery at affected part | |

[0031] It is observed from the above table that the external remedy of Shikonin compounds is suitable for treatment of most abscess, wound, scabies and herpes; the effect is prominent for burn and scalding without cicatrices after recovery.

[0032] Table 3 shows the animal test results for using Shikonin, 1, 1'-dimethylacrylshikonin and Acetylshikonin to restrain tumor.

Table 3

| Type of tumour | 1, 1'-dimethylacrylshikonin | | Acetylshikonin | | Shikonin | |
|---------------------------|-----------------------------|---------------------|-----------------------|---------------------|-----------------------|---------------------|
| | Tumor inhibitory rate | Life prolonged rate | Tumor inhibitory rate | Life prolonged rate | Tumor inhibitory rate | Life prolonged rate |
| Ascitic tyre liver cancer | | 113.4% | 47.8% | 112.6% | | 130.8% |

Table 3 (continued)

| Type of tumour | 1,1'-dimethylacrylshikonin | | Acetylshikonin | | Shikonin | |
|-------------------|----------------------------|---------------------|-----------------------|---------------------|-----------------------|---------------------|
| | Tumor inhibitory rate | Life prolonged rate | Tumor inhibitory rate | Life prolonged rate | Tumor inhibitory rate | Life prolonged rate |
| S180 | 9.63% | | 35.7% | | | |
| Lewis lung cancer | 42.8% | | 52.6% | | | |
| L1210 | | | | 128% | | |
| W256 | | | 77% | | | |

[0033] It is observed from the above table that 1,1'-dimethylacrylshikonin has different extent of therapeutic effect for liver cancer, S180 and Lewis lung cancer; Acetylshikonin has different extent of therapeutic effect for liver cancer, S180, L1210 and Lewis lung cancer and W256; Shikonin is only effective for liver cancer. As is shown in the experiment using Shikonin compounds to restrain virus, oral dosing 1,1'-dimethylacrylshikonin is made on the 7th day since duck is infected by DHBV with 100mg/kg and twice a day, the inhibitory effect for DHBV-DNA level in blood serum of infected duck is prominent in 10 days ($P<0.05-0.01$) without toxic reaction; for the 50mg/kg group, significant inhibitory effect is shown ($P<0.05$).

[0034] As is shown in the experiment using 1,1'-dimethylacrylshikonin to restrain HBV, if concentration is 30 μ g/ml, average inhibitory rate for HBsAg is 96.2601% and for HBeAg is 91.6056%. Table 4 shows the invitro test results of Shikonin and 1,1'-dimethylacrylshikonin resisting HIV-1 reverse transcriptase and integrase.

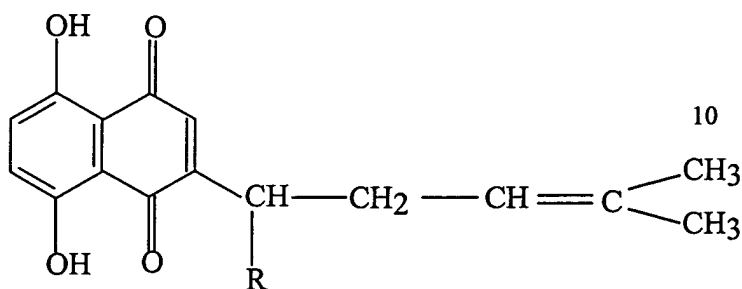
Table 4

| | | IC ₅₀ (μ g/ml) |
|-------------------------------------|-------------------------|--------------------------------|
| Resisting HIV reverse transcriptase | Positive control PFA | 0.097 |
| | Shikonin compounds | >20 |
| Resisting HIV integrase | Positive control ABPS-Y | 0.922 |
| | Shikonin compounds | 12.467 |

[0035] Study the effect of Shikonin compounds with the model of mouse's low immunologic function caused by mitomycin C. If 6mg/kg 1,1'-dimethylacrylshikonin is injected in the abdominal cavity for 5 days without interruption, the cell toxicant in the mouse's splenic cell and NK cell increases by around 20% ($P<0.001$), which indicates that 1,1'-dimethylacrylshikonin can recover the injury of intraperitoneal macrophage, improve the migration ability of intraperitoneal macrophage, raise the activity of T lymphocytes, and promote the immune response of T lymphocytes, enhance the nonspecific immunity and specific cell immunity effect of body.

Claims

1. A medicament for prevention or treatment of microorganism infection in human body, inflammation, malignant tumor, hemorrhage, hematopathy and autoimmune disease, wherein the medicament contains 1 to 5 of Shikonin compounds and its salt shown in formula (1),



Wherein R is a group selected from H, OH, $(\text{CH}_3)_2\text{C}=\text{CHC}(\text{O})\text{O}-$, $\text{CH}_3\text{C}(\text{O})\text{O}-$, $(\text{CH}_3)_2\text{C}=\text{C}(\text{CH}_3)\text{CH}_2\text{C}(\text{O})\text{O}-$, $(\text{CH}_3)_2\text{COHCH}_2\text{C}(\text{O})\text{O}-$ and $(\text{CH}_3)_2\text{C}[\text{OC}(\text{O})\text{CH}_3]\text{CH}_2\text{C}(\text{O})\text{O}-$.

2. The medicament as stated in claim 1, wherein R is 1 to 3 groups selected from OH, $(\text{CH}_3)_2\text{C}=\text{CHC}(\text{O})\text{O}-$ and $\text{CH}_3\text{C}(\text{O})\text{O}-$.
3. The medicament as stated in claim 1, wherein R is $(\text{CH}_3)_2\text{C}=\text{CHC}(\text{O})\text{O}-$ and/or $\text{CH}_3\text{C}(\text{O})\text{O}-$.
4. The medicament as stated in claim 3, wherein R is $(\text{CH}_3)_2\text{C}=\text{CHC}(\text{O})\text{O}-$.
5. The medicament as stated claim 1, wherein the purity of each compound is 80% or more.
6. The medicament as stated in claim 1, wherein the purity of each compound is 90% or more.
7. The medicament as stated in claim 1, wherein the effective components are 70% or more when the medicament contains more than one compound.
8. The medicament as stated in claim 1, wherein the medicament further contains other active components.
9. The medicament as stated in claim 1, wherein the medicament is used for treatment or prevention of microogomism infections of every system of human body which include stuptocowus pneumonia klilsiella hiicotacter Rylari candia cryptococcus dermatophyte every kind of mycoplarma infoctionsinclude mycoplarma pneumonia: every kind of chlamgdia infection or virus infection include hepaltities virus. Influenta virus, herpes virus and HIV virus.
10. The medicament as stated in claim 1, wherein the medicament is used for treatment or prevention of cancers associated with hydroperitoneum tumour such as liver cancer and L1210, and entity tumor such as sarcoma 180, stomach cancer 823, squama carcinoma 109 or lung cancer.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00138

A. CLASSIFICATION OF SUBJECT MATTER

A61K31/122 A61P31/00, 7/00, C07C50/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | Lin, Zhibin et al, "Study on anti-inflammatory action of chemical composition of shikonin" Beijing Yixueyuan Xuebao (1980), 12(2), 101-105, See the whole document | 1-10 |
| X | CN1253972A 24.May 2000, See the whole document | 1-10 |
| A | CN1117525A 28.Feb 1996, See the whole document | 1-10 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

| | |
|--|--|
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier application or patent but published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

| | |
|---|--|
| Date of the actual completion of the international search 12.01.2004 | Date of mailing of the international search report 22 · JAN 2004 (22 · 01 · 2004) |
| Name and mailing address of the ISA/CN 6 Xitucheng Rd., Jimen Bridge, Haidian District, 100088 Beijing, China Facsimile No. 86-10-62019451 | Authorized officer Telephone No. 86-10-62085607 |

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family membersInternational application No.
PCT/CN03/00138

| Patent document Cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| CN1253972A | 24-05-2000 | None | |
| CN 1117525A | 28-02-1996 | None | |

Form PCT/ISA /210 (patent family annex) (July 1998)